**TITLE PAGE**

**Title:**

Unmasking the hidden tuberculosis mortality burden in a large postmortem study in Maputo Central Hospital, Mozambique

**Authors:**

Alberto L Garcia-Basteiro[1,2,3]*, Juan Carlos Hurtado[2,4]*, Paola Castillo [2,5]*, Fabiola Fernandes[6], Mireia Navarro[4], Lucilia Lovane[4] , Isaac Casas[4], Llorenç Quintó[1], Dercio Jordao[6], Mamudo R Ismail[6], Cesaltina Lorenzoni[6], Carla Carrilho[6], Ariadna Sanz[2], Natalia Rakislova[5], Aurea Mira[7], Miriam J Alvarez-Martínez[2,4], Anelsio Cossa[1], Frank Cobelens[3], Inácio Mandomando[1,7], Jordi Vila[2,4], Quique Bassat[1,2,9,10#], Clara Menendez[1,2,11#], Jaume Ordi [2,5#], Miguel J. Martínez [2,4#]

[#] Contributed equally to this work and share senior authorship.

* Contributed equally to this work and share primary authorship.

**Affiliations:**

[1]Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique.

[2]ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain.

[3]Amsterdam Institute for Global Health and Development, Amsterdam University Medical Centers, Amsterdam, The Netherlands.

[4]Department of Microbiology, Hospital Clinic, Universitat de Barcelona, Spain.

[5]Department of Pathology, Hospital Clinic, Universitat de Barcelona, Spain.

[6]Department of Pathology, Faculty of Medicine/Eduardo Mondlane University and Maputo Central Hospital, Maputo, Mozambique.

[7]Biomedical Diagnostic Centre (CDB), Hospital Clínic, University of Barcelona, Barcelona, Spain.

[8]Instituto Nacional de Saúde (INS), Ministério da Saúde, Maputo, Moçambique

[9]ICREA, Catalan Institution for Research and Advanced Studies, Pg. Lluís Companys 23, 08010 Barcelona, Spain

[10]Pediatric Infectious Diseases Unit, Pediatrics Department, Hospital Sant Joan de Déu (University of Barcelona), Barcelona, Spain

[11]Consorcio de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain.

**Corresponding author:**

Miguel J. Martínez

Department of Microbiology, Hospital Clinic, Universitat de Barcelona, Spain

Barcelona Institute for Global Health, Spain

myoldi@clinic.ub.es

**Take home message:**

This study shows the usefulness of molecular assays in ascertaining TB diagnosis at death. It questions the information of clinical diagnoses obtained from hospital registries as a reliable tool for TB mortality estimation.

**Running head:** Burden of TB in a large postmortem study in Mozambique.

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**Abstract**

Sensitive tools are needed to accurately establish the diagnosis of tuberculosis (TB) at death, especially in low-income countries. The objective of this study was to evaluate the burden of TB in a series of patients who died in a tertiary referral hospital in sub-Saharan Africa using an in-house real time PCR (TB-PCR) and the Xpert MTB/RIF Ultra (Xpert Ultra) assay.

Complete diagnostic autopsies were performed in a series of 223 deaths (56.5% being HIV-positive), including 54 children, 57 maternal deaths and 112 other adults occurring at the Maputo Central Hospital, Mozambique. TB-PCR was performed in all lung, cerebrospinal fluid and central nervous system samples in HIV-positive patients. All samples positive for TB-PCR or showing histological findings suggestive of TB were analysed with the Xpert Ultra assay.

TB was identified as the cause of death in 31 patients: 3/54 (6%) children, 5/57 (9%) maternal deaths and 23/112 (21%) other adults. The sensitivity of the main clinical diagnosis to detect TB as the cause of death was 19.4% (95% CI: 7.5-37.5) and the specificity was 97.4% (94.0-99.1) compared to autopsy findings. Concomitant TB (TB disease in a patient dying of other causes) was found in 31 additional cases. Xpert Ultra helped to identify 15 cases of concomitant TB. In 18 patients, *M. tuberculosis* DNA was identified by TB-PCR and Xpert Ultra in the absence of histological TB lesions. Overall, 62 cases (27.8%) had TB disease at death and 80 (35.9%) had TB findings.

The use of highly sensitive, easy to perform molecular tests in complete diagnostic autopsies may contribute to identifying TB cases at death that would have otherwise been missed. Routine use of these tools in certain diagnostic algorithms for hospitalised patients needs to be considered. Clinical diagnosis showed poor sensitivity for the diagnosis of TB at death.

*Keywords:* tuberculosis; epidemiology; mortality; post-mortem; Xpert Ultra; minimally invasive autopsy; diagnosis

## Introduction

Tuberculosis (TB) remains a major public health concern in most countries of the world. In 2017, the World Health Organization (WHO) estimated around 10 million new cases and 1.6 million deaths attributable to TB.[1] As a single cause of death (CoD), TB is the main infectious killer at a global level. It is also the most frequent cause of HIV-associated deaths[2], and ranks among the principal CoD among women of reproductive age[3–5]. Similar to other countries in the region, the HIV and TB epidemic in Mozambique is devastating.[1,6] The estimated national incidence rate of TB in 2017 was 551 per 100,000 inhabitants with a case fatality ratio of 31% (around 49,000 deaths in 2017) and HIV coinfection rate among new TB cases of 40%.[1]

Accurate and reliable TB mortality data are fundamental to improve patient management, prioritize public health interventions and assess progress in the WHO End TB strategy indicators[7]. Despite the enormous TB burden, there is considerable uncertainty as to the actual mortality attributable to this disease, especially in some low-income countries (LICs) with high disease burden.[8] Mortality estimates based on case fatality rates reported by national TB programmes are of low quality.[9] Clinical diagnosis and verbal autopsies have limited sensitivity and specificity for diagnosing TB as the CoD compared to complete diagnostic autopsy (CDA), the current gold standard[2,9–13]. Studies assessing clinico-pathological discrepancies have shown a high degree of misclassification when assigning deaths to TB in either direction (clinically missed TB causing death and false attribution of TB as the CoD when not present).[10,11]

Additionally, most of the TB disease identified in CDA studies is considered as responsible for the death of the patients[2]. The diagnosis of TB disease in these studies is based on obvious macroscopic disease, confirmed microscopically by the presence of granulomas with Ziehl-Neelsen stain-positive bacilli. However, it is likely that earlier forms of TB, in which pathological findings might not be that obvious, are missed. When there are other plausible causes of death and concomitant TB disease, the exact

role that TB might have played in the chain of events leading to death may not be clear. This can be especially true in immunocompromised HIV-positive patients.[14]

As part of a large post-mortem study conducted at a tertiary referral hospital in Mozambique [15–18] two molecular tests were used to diagnose TB, an in house real time PCR and the Xpert MTB/RIF Ultra assay (Xpert Ultra). The study had two main objectives: 1) to describe the overall burden of TB as the CoD and as a concomitant finding (TB disease identified at death, but not directly causing death), and 2) to assess the proportion of clinically missed TB cases.

## Methods

### Study design

This was an ancillary study to a prospective observational postmortem evaluation aimed at validating minimally invasive autopsy for CoD determination in different age groups compared withCDA, the gold standard technique.[19] This study included childhood (≥ 1 months to 15 years old), adult, and maternal deaths (the latter defined as deaths among women while pregnant or within 42 days of termination of pregnancy). Traumatic deaths were excluded.[20] The study was conducted from November 2013 to March 2015 at the Department of Pathology of the Maputo Central Hospital, a 1500-bed government-funded tertiary health care centre, in collaboration with the departments of paediatrics, internal medicine and obstetrics and gynaecology. Prior informed consent was obtained from the relatives of the deceased. The study received approval from the Clinical Research Ethics Committee of the Hospital Clinic of Barcelona (Spain; File 2013/8677) and the National Bioethics Committee of Mozambique (Mozambique; Ref. 342/CNBS/13).

The pathological and microbiological methods of the CDA procedures have been described elsewhere.[21,22], Samples were obtained from all organs for histological and microbiological analysis. Samples for microbiological testing were collected in nucleic-

acid preserving buffer (ATL lysis buffer, Qiagen). Clinical information was collected from each patient using a standardized questionnaire after thorough revision of the entire medical record. The data obtained included demographic data, past medical history, as well as information about the inpatient admission process, signs and symptoms, physical examination, laboratory and imaging results when available, and treatment received during hospitalisation. For maternal deaths, the obstetric history was also reviewed. Following analyses of the CDA samples, a panel composed of a pathologist, a microbiologist and a clinician (paediatrician, internist or gynaecologist, depending on the age group) evaluated the pathological and microbiological reports of the CDA and the clinical data and assigned the CDA diagnosis of CoD. The main results of this validation project have been published.[15–18]

HIV status was confirmed postmortem by an automated method detecting antibodies against HIV (ADVIA Centaur HIV 1/0/2 Enhanced assay, Siemens Healthcare Diagnostics) and by viral load testing using the Cobas TaqMan HIV-1 test v2.0 (Roche Molecular Systems).

*TB testing strategy and laboratory procedures*

The TB testing strategy of the study is summarized in **Figure 1**. The initial microbiological diagnosis of TB was performed using an in-house real-time PCR targeting *M. tuberculosis* (TB-PCR). TB-PCR was performed in all lung samples obtained at CDA, independently of the presence or absence of histological lesions, and in any other organ showing histological lesions suggestive of TB (granulomatous inflammatory reaction and/or caseous necrosis). In addition, in HIV positive patients, TB was routinely tested by TB-PCR in all CNS and CSF samples (independently of the presence or absence of lesions). Histological testing for TB included Ziehl-Neelsen staining when TB was suspected on the hematoxylin-eosin (H&E) stained slides.

In addition, the Xpert MTB/RIF Ultra assay (hereinafter Xpert Ultra) was performed in: a) any organ with histological lesions suggestive or compatible with TB and positive TB-PCR (cases with TB disease), and b) in all deaths in which there was microbiological-histological discordance (histological lesions suggestive of TB with negative TB-PCR or positive TB-PCR without histological lesions suggestive of TB).

Tissue samples for Xpert Ultra were thawed and homogenized using a handheld rotor-stator homogenizer (Qiagen) in ATL lysis buffer (Qiagen). One-hundred µL of the homogenized tissue sample were added to 300 µL of saline solution. The resulting 400 µL of sample were mixed with 1600 µL of Xpert Ultra Sample Reagent and then loaded into the cartridge. In house TB-PCR was performed using the procedures described by Espasa *et al*.[23]

*Definitions of TB associated findings*

*"Histological lesions suggestive of TB"* were defined as granulomatous inflammatory reactions with or without caseous necrosis, independently of the presence or absence of visible acid-fast bacilli on Ziehl-Neelsen staining. "*Histological lesions compatible with TB"* included non-specific inflammatory reactions (such as neutrophilic inflammation) which have been described in TB.[24]

*"TB disease at death"* included histological lesions suggestive of TB with a positive TB-PCR or a positive Xpert Ultra in any sample or when there were TB-compatible histological lesions and both TB-PCR and Xpert Ultra were positive. TB disease at death was further classified as "*TB as CoD"* when the review of the entire CDA (including histological, microbiological and clinical data) following a previously described algorithm of CoD determination, deemed TB to be the CoD, [25] which corresponds to the "-a diagnosis" in the WHO international form of medical certificate of death.[26] A case was classified as "*concomitant TB"* when histological lesions

compatible with TB were present at death but the review of the CDA deemed another disease as the most likely CoD ("-b", "-c diagnoses", or "other significant conditions" in the WHO international form of medical certificate of death). Finally, a case was classified as "*M. tuberculosis DNA detection"* when both TB-PCR and Xpert Ultra were positive in the absence of compatible histological findings. A single positive TB-PCR without any histological finding compatible with TB was considered a false positive result, since sample contamination or a true false positive result due to assay- or human-related performance could not be ruled out.

*Statistical analysis*

All the clinical data and the results of the histopathological and microbiological examination of the samples were analysed using Stata 13 (Stata Corp., College Station, TX). We calculated the proportions of: a) cases in which TB was the CoD; b) cases with concomitant TB; and c) cases with *M. tuberculosis* DNA. We estimated the sensitivity, specificity and predictive values of the clinical diagnoses to detect TB disease as the CoD (when the clinician specified TB as the main diagnosis as well as to detect concomitant TB (when the clinician specified TB among the diagnoses or initiated anti-TB treatment), using CDA diagnoses as the gold standard.

## Results

The analysis included 223 deaths: 54 children, 57 maternal deaths and 112 other adults. HIV infection was identified in 32.7% (17/52), 65.2% (36/57) and 63.2% (73/112) of these deaths, respectively (56.5% of the overall series).

*Tuberculosis-associated findings*

TB was diagnosed as the CoD in 31 patients: 3/54 children (5.6%, 95% confidence interval [CI]: 1.2-15.4), 5/57 maternal deaths (8.8%, 95% CI: 2.9-19.3), and 23/112 other adults (20.5%, 95% CI: 13.5-29.2). Among HIV-positive cases, TB was identified

as the CoD in 0/17 (0%) children, 3/34 (8.8%, 95% CI: 1.9-23-7) maternal deaths, and 18/73 (24.6%, 95% CI: 15.3-36.1) other adults. The most frequent form of TB as the CoD was miliary TB, (23/31; 74.2%), followed by pulmonary TB (6/31; 19.4%) and TB meningitis (2/31; 6.5%) (**table 1).** Among the 31 patients dying of TB, Xpert Ultra tested positive in all lung samples and 14 CSF samples.Concomitant TB was identified in 31 additional patients: 8/54 children (14.8%), 18/112 adults (13.9%) and 5/57 maternal deaths (8.8%). Of these, 22 had pulmonary TB, 5 disseminated TB (more than 1 organ involved) and 4 had extrapulmonary TB involving a single organ (1 case with splenic TB, 3 with TB meningitis). **Table 2 and figure 2** show the CoD of the cases with concomitant TB. Initially, 15 of 31 (48.4%) cases of concomitant TB disease did not have histological lesions suggestive of TB. Only after Xpert Ultra results were available (which prompted further histological review) the histological findings were deemed as TB compatible lesions (mostly inflammatory lesions).

Eighteen additional cases fulfilled the definition of "*M. tuberculosis* DNA detection": 3/54 (6.6%) children, 5/57 (8.6%) maternal deaths and 10/112 (8.9%) adults. Of these cases, six died of an infectious cause and 12 of non-infectious conditions. Of the 18 cases in which only *M tuberculosis* DNA was detected, six (33.3%) were HIV positive, and one (5.6%) had a history of past TB.

Overall, TB findings were confirmed in 80 cases: 14/54 children (25.9%), 15/57 (26.3%) maternal deaths and 51/112 (45.5%) other adults. Among HIV positive patients, TB was confirmed in 3/17 (17.6%), 14/36 (38.9%) and 37/74 (50.7%) of patients in each study group, respectively (**Table 1**). Rifampicin resistance was detected by Xpert Ultra in eight cases.

*Clinical characteristics of patients with TB findings*

A clinical history of TB was reported in none of the children, in 2 (3.5%) maternal deaths and in 16 (14.3%) adults. Twenty of 31 (65%) patients with TB as the CoD had

reported cough compared to nine of 31 (29%) among those with concomitant TB at death (p=0.005). Among patients with TB findings, 8 cases were on antiTB treatment prior to admission, and in 10 antiTB treatment was initiated during admission. No differences were observed in terms of fever at admission, between patients with TB as the CoD and those with concomitant TB (17/31, 55%; vs. 19/31, 61%, p=0.6). The characteristics of cases with TB disease as the CoD, with concomitant TB, with *M. tuberculosis* DNA detection and those without any TB finding are shown in **table 3.**

*Clinico-pathological discrepancies*

Assuming that the first (main) clinical diagnosis was the CoD for clinicians, they had considered TB disease as the CoD in 11 of the 223 (4.9%) patients). However, the clinical diagnosis of TB as the CoD was correct in only 6 patients **(figure 2)**. Thus, the main clinical diagnosis had a sensitivity to detect TB disease as CoD of 19.4% (6/31, 95% CI: 7.5-37.5) and a specificity of 97.4% (95% CI: 94.0-99.1) **(table 4**). Among the six cases in which clinicians correctly specified TB as the main clinical diagnosis, complete agreement with the form or localisation was only confirmed in two cases of pulmonary TB. Four patients showing miliary TB in the CDA were diagnosed as pulmonary TB or TB meningitis (3 and 1 patient respectively).

Clinicians specified tuberculosis as one of the diagnoses at death (or initiated TB treatment) in 36 cases. However, only 20 cases were correctly diagnosed. The sensitivity of the clinical diagnosis (including decision to treat TB) for diagnosing TB disease at death (regardless of whether it was the CoD or concomitant TB) was 32.3% (20/62, 95% CI: 20.9 - 45.3) with a specificity of 90.1% (95% CI: 84.1-94.2). Thus, TB disease at death remained undiagnosed in 68% (42/62) of cases. By study group, the sensitivity of any clinical diagnosis to detect TB disease at death was 27.3% (3/11) in children, 24.4% (10/41) in adults and 10.0% (2/10) in maternal deaths (**table 4**).

## Discussion

This study, which is part of one of the largest autopsy studies conducted in sub-Saharan Africa, demonstrates the enormous burden of tuberculosis among children and adults, including maternal deaths, dying in a reference hospital in Mozambique. An even higher burden was found among HIV-positive adults and maternal deaths, in whom the proportion of TB findings rose up to 51% and 39% respectively. Importantly, it also shows alarming proportions of TB disease missed by clinicians and highlights the limitations of clinical diagnosis for ascertaining TB in resource constrained settings. In addition, the use of molecular assays (this is the first time Xpert Ultra has been used in a postmortem study) allowed *M. tuberculosis* to be detected in 8% (18/223) of patients in whom no histological changes were identified, possibly reflecting early forms of TB. This indicates that the total burden of TB at death might be even higher than what has been reported in clinical and epidemiological and even in many autopsy studies.

A high burden of TB has also been found in some autopsy studies in countries neighbouring Mozambique, also within the context of high HIV and TB burden. In Zambia 65% of deaths in hospitalised patients >16 years of age (81% HIV-positive) were due to TB and in Kwazulu Natal, South Africa, 50% of adult inpatient deaths aged 20-45 years had culture-confirmed TB (96% HIV-positive).[10,27] A meta-analysis of autopsy studies showed that the prevalence of TB among HIV-positive adult deaths in sub-Saharan Africa was 43.2% (95% CI 38.0– 48.3%).[2] We found a slightly lower percentage of cases in which TB was the CoD, perhaps because of different HIV treatment status, or because in some cases TB findings were not deemed as the most likely CoD. In fact, in their metaanalysis Gupta *el al,* reported that in 91.4% (95% CI: 85.8–97.0%) of cases in which TB was present at death it was also the primary CoD. Interestingly, we found that TB was the CoD in 50% of cases in which TB disease was present at death. This considerable proportion of TB disease not causing death can

largely be explained by the additional cases with concomitant TB that were captured by a detailed pathological evaluation and the use of TB-PCR and Xpert Ultra in the diagnostic algorithm, and which might have been missed in other studies using traditional diagnostic tools.

Additionally, in 18 cases with no histological evidence of TB, *M. tuberculosis* DNA was detected by two different molecular methods. These findings make the classification of this form of TB especially difficult, since the postmortem diagnosis of TB is not based on the diagnostic criteria used in living patients (sputum sample, presence of symptoms or chest radiography). It is unlikely that these findings correspond to contamination in the autopsy room, since disposable materials are used in each autopsy and the time that the tissue samples were exposed to potential airborne contamination is limited. Neither is it likely that these are false-positive cases, since DNA was detected by two different assays with different molecular targets. These findings may represent cases of incipient TB in which small histological lesions might be present but missed, since not all the tissue from all the organs was sampled for histological analysis.[28] However, these *M. tuberculosis* DNA findings may also represent the increasingly controversial concept of latent TB infection. Although it has been postulated that TB infection cannot be detected through direct diagnostic methods[29], it has been suggested that it might be detected through detailed molecular studies in cases without histological evidence.[30] It has also been suggested that there could be periods in the so-called latency or unstable infection period during which *M. tuberculosis* replicates at a higher rate, but this replication remains self-controlled.[14] The present study suggests and supports the idea of the existence of a spectrum of TB disease, ranging from early forms of TB in which few bacilli are present (that might not necessarily develop into TB disease) to the traditional patent TB lesions (**Figure 3**).

Clinical misclassification of TB was very frequent. In over 80% of TB identified as the CoD in CDA, the clinicians failed to identify TB as the event leading to death and

overall, 67% of cases with TB disease at death were missed by the clinicians. Conversely, in 45% of the cases clinicians incorrectly established TB as the main cause of death or the presence of TB disease at death. The clinico-pathological discrepancies of TB disease at death have previously been reported and have implications for patient management as well as for estimating TB mortality burden.[9,10] Clinical diagnosis is not a good proxy of mortality by TB, and contrarily to what it was expected, it was poor in both HIV-positive and HIV-negative cases with TB disease. Thus, it is likely that the results of verbal autopsies are similarly bad or even worse as a tool to quantify TB mortality.[12,13,31,32] Nonetheless, more studies comparing verbal autopsies with CDA are needed in order to demonstrate their usefulness as a tool for assessing TB?? mortality burden. It was of note that out of the 36 cases with TB findings in whom clinicians specified TB as a clinical diagnosis, only 18 were started on antiTB treatment (prior to or during admission). Possible reasons for this may be that when the patient died, clinicians might have reconsidered the potential diagnoses of the patient and then added TB, or that patients might have been too ill or died very early during admission. This which might have led to antiTB treatment not being initiated. Poor reporting of antiTB treatment initiation cannot be discarded. Ç

Our study has several limitations. First, our conclusions can only be generalised to hospitalised patients of settings with similar epidemiological characteristics and do not necessarily represent the contribution of TB to all population-based mortality in Maputo or Mozambique. The likelihood of being hospitalised depends on access to healthcare, health infrastructure, severity of disease determined by healthcare workers and severity perceived by patients and relatives, among other sociological and behavioural factors. Likewise, diagnostic discrepancies might occur less frequently in larger hospitals, thus, those found in this study (carried out at the main reference hospital of the country) might underestimate the number of clinic-pathological discrepancies in general Mozambican health facilities. Second, as mentioned previously, all the organ

samples were analysed histologically, but not all in their entirety, thus some cases of concomitant TB might have been missed. Third, TB culture was not contemplated in the study design, and this method could have helped to better characterise the microbiological findings. Likewise, the use of Xpert Ultra as a screening tool in all cases could have determined a higher number of confirmed cases and allowed estimation of the added yield of the use of this highly sensitive technology. Lastly, the quality of the clinical information may have been suboptimal in some cases of hospitalised patients with severe disease status, and their degree of consciousness might have made some of the results prone to information bias. Thus, despite comprehensive evaluation of all the clinical records available, some relevant information about the medical history might have been missed by patients or relatives.

In conclusion, we found a high burden of TB disease at death (as CoD and as concomitant disease) in all groups studied. The use of highly sensitive molecular tests in CDA helped to identify cases of TB disease at death that would have otherwise been missed. Indeed, the results demonstrate that in our setting, clinical diagnoses miss most of the TB disease which is detected in CDA. In addition, Xpert Ultra may have the potential to identify earlier forms of TB, before histological lesions are evident, or potentially, unstable latent TB infection.

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**Figure Legends**

**Figure 1.** Algorithm for determination of TB diagnosis used in samples from complete diagnostic autopsies.

**Figure 2.** Alluvial diagram showing cause of death (CoD) as assigned in the complete diagnostic autopsy (CDA) and per clinical diagnosis whenever a TB diagnosis was involved. The left column shows all the TB diagnoses (as CoD) in CDA (31) as well as the associated CDA diagnoses when clinicians assigned TB as the CoD (5). The right column shows the clinical diagnoses of CoD when TB was specified by clinicians as well as the associated clinical diagnosis when the results of the CDA assigned TB as the CoD.

**Figure 3.** Natural history of the TB model depending on bacillary burden and the likelihood of having immunological, microbiological, histological or radiological evidence at the time of death.

*Adapted from a model from Esmail *et al.*[14]

**

**Table 1.** Number of patients with tuberculosis (TB) disease as the CoD, with concomitant TB disease and with *M tuberculosis* detection at death by study group and among HIV positive cases.

| | | TB disease at death (compatible histology and confirmed with in-house PCR and Xpert Ultra) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TB as cause of death (CoD)[1] | | Concomitant TB[2] | | | | *M tuberculosis* DNA detection[3] | | Total cases with TB findings[4] | |
| **Study group** | **Autopsies** | **n** | **%** | **n** | **%** | **Total** | **%** | **n** | **%** | **#** | **%** |
| *All autopsies* | | | | | | | | | | | |
| **Children** | **54** | 3 | 5.6 | 8 | 14.8 | 11 | 20.4 | 3 | 5.6 | 14 | 25.9 |
| **Maternal Deaths** | **57** | 5 | 8.8 | 5 | 8.8 | 10 | 17.5 | 5 | 8.8 | 15 | 26.3 |
| **Adults** | **112** | 23 | 20.5 | 18 | 16.1 | 41 | 36.6 | 10 | 8.9 | 51 | 45.5 |
| **Total** | 223 | 31 | 13.9 | 31 | 13.9 | 62 | 27.8 | 18 | 8.1 | 80 | 35.9 |
| *Autopsies among HIV positive cases** | | | | | | | | | | | |
| **Children** | **17** | 0 | 0.0 | 3 | 17.6 | 3 | 17.6 | 0 | 0.0 | 3 | 17.6 |
| **Maternal Deaths** | **36** | 5 | 13.9 | 4 | 11.1 | 9 | 25.0 | 5 | 13.9 | 14 | 38.9 |
| **Adults** | **73** | 18 | 24.7 | 13 | 17.8 | 31 | 42.5 | 6 | 8.2 | 37 | 50.7 |
| **Total** | 126 | 23 | 18.3 | 20 | 15.9 | 43 | 34.1 | 11 | 8.7 | 54 | 42.9 |

[1]TB as Cause of death (CoD): when review of the entire CDA (including histological, microbiological and clinical data) following a previously described algorithm of CoD determination deemed TB to be the CoD, This definition required the presence of histological TB-compatible lesions and microbiological confirmation of TB by molecular assays.

[2]Concomitant TB when histological lesions compatible with TB were present at death but review of the CDA deemed another disease as the most likely cause. This definition required the presence of histological TB-compatible lesions and microbiological confirmation of TB by molecular assays.

[3]*M. tuberculosis* DNA detection was attributed when both TB-PCR and Xpert Ultra were positive in the absence of compatible histological findings.

[4]TB findings. In this column we include all cases in which TB disease was found at death (as the cause of death or as a concomitant finding) and cases in which only *M. tuberculosis* DNA were detected.

*HIV status of two cases in children could not be ascertained.

**Table 2.** Main cause of death of patients with concomitant TB (n=31).

| # | Group | HIV status | CDA A diagnosis | Infectious agent identified | Type of TB (organs affected) |
|---|---|---|---|---|---|
| 1 | Children | Negative | Rabies | Rabies virus | Pulmonary TB |
| 2 | Children | Negative | Peritonitis | Unspecified | Pulmonary TB |
| 3 | Children | Negative | Malignant brain tumour | | Pulmonary TB |
| 4 | Children | Positive | Sepsis | *Streotococcus pneumoniae* | Pulmonary TB |
| 5 | Children | Negative | Meningoencephalitis | *Cryptococcus gattii* | Extrapulmonary TB (CNS) |
| 6 | Children | Positive | Meningitis | *Streptococcus pneumoniae* | Pulmonary TB |
| 7 | Children | Positive | Pneumocystosis | *Pneumocystis jirovecii* | Extrapulmonary TB (CNS) |
| 8 | Children | Negative | Sepsis | *Streptococcus pneumoniae* | Pulmonary TB |
| 9 | Maternal deaths | Positive | Meningoencephalitis | *Cryptococcus neoformans* | Extrapulmonary TB (spleen) |
| 10 | Maternal deaths | Positive | Septic abortion | *Mycoplasma hominis* | Pulmonary TB (miliary TB, both lungs) |
| 11 | Maternal deaths | Positive | Encephalitis | Unspecified | Pulmonary TB |
| 12 | Maternal deaths | Positive | Puerperal sepsis | | Pulmonary TB |
| 13 | Maternal deaths | Negative | Pneumonia | Unspecified | Pulmonary TB |
| 14 | Other adults | negative | Mucormycosis | Rhizopus oryzae | Extrapulmonary TB (CNS) |
| 15 | Other adults | Positive | Diffuse large B-cell lymphoma | | Pulmonary TB |
| 16 | Other adults | Positive | Intracerebral haemorrhage | | Pulmonary TB |
| 17 | Other adults | Positive | Sepsis | *Streptococcus dysgalactiae* | Pulmonary TB |
| 18 | Other adults | Positive | Pneumonia | *Klebsiella pneumoniae* | Disseminated TB (liver lung) |
| 19 | Other adults | Positive | Diffuse large B-cell lymphoma | | Pulmonary TB |
| 20 | Other adults | Negative | Meningitis | Herpes simplex virus type 1 | Pulmonary TB |
| 21 | Other adults | Positive | Sepsis | *Escherichia coli* | Pulmonary TB |
| 22 | Other adults | Negative | Sepsis | *Candida glabrata* | Pulmonary TB |
| 23 | Other adults | Positive | Disseminated Kaposi's sarcoma | *Human herpesvirus 8* | Pulmonary TB |
| 24 | Other adults | Negative | Sepsis | *Enterobacter spp.* | Pulmonary TB |
| 25 | Other adults | Positive | Pneumonia | *Pseudomonas aeruginosa* | Disseminated TB (lung, CNS) |
| 26 | Other adults | Positive | Toxoplasmosis | *Toxoplasma gondii* | Disseminated TB (lung, liver, spleen) |
| 27 | Other adults | Negative | Cardiac arrest | | Pulmonary TB |

| | | | | | |
|---|---|---|---|---|---|
| 28 | Other adults | Positive | Toxoplasmosis | *Toxoplasma gondii* | Pulmonary TB |
| 29 | Other adults | Positive | Sepsis | *Legionella pneumophila* | Disseminated TB (spleen, lung) |
| 30 | Other adults | Positive | Meningitis | *Streptococcus pneumoniae* | Disseminated TB (lung, CNS) |
| 31 | Other adults | Positive | Pneumonia | Unspecified | Pulmonary TB |

CNS: central nervous system

**Table 3.** Characteristics of cases in whom the case of death was TB, with concomitant TB disease at death and with *M. tuberculosis* detection without histological evidence of TB.

| Characteristics | TB as cause of death (n=31) n (%) | Concomitant TB (n=31) n (%) | *M tuberculosis* DNA detection (n=18) n (%) | Others (no TB findings) (n=143) n (%) | Total (n=223) n (%) |
|---|---|---|---|---|---|
| **Sex** | | | | | |
| Male | 13 (41.9) | 20 (64.5) | 7 (38.9) | 55 (38.5) | 95 (42.6) |
| Female | 18 (58.1) | 2 (35.5) | 11 (61.11) | 88 (61.5) | 128 (57.4) |
| **Study Group** | | | | | |
| Children | 3 (9.7) | 8 (25.8) | 3 (16.7) | 40 (28.0) | 54 (24.2) |
| Maternal deaths | 5 (16.1) | 5 (16.1) | 5 (27.8) | 42 (29.4) | 57 (25.6) |
| Adults | 23 (74.2) | 18 (58.1) | 10 (55.6) | 61 (42.7) | 112 (50.2) |
| **HIV status**[#] | | | | | |
| Positive | 23 (76.7)[#] | 20 (64.5) | 6 (35.3)[#] | 71 (49.7) | 95 (43.0) |
| Negative | 7 (23.3)[#] | 11 (35.5) | 11 (64.7)[#] | 72 (50.3) | 126 (57.0) |
| **History of TB** | | | | | |
| Yes | 6 (19.4) | 4 (12.9) | 1 (5.6) | 7 (4.9) | 18 (8.1) |
| No | 25 (80.6) | 27 (87.1) | 17(94.4) | 136 (95.1) | 105 (91.9) |
| **On anti TB treatment prior toadmission** | | | | | |
| Yes | 4 (13.3)* | 4 (12.9) | 0 (0.0)* | 6 (4.4)* | 14 (6.4) |
| No | 26 (86.7)* | 27 (87.1) | 17 (100)* | 136 (95.6)* | 206 (93.6) |
| **Reported fever at admission**[&] | | | | | |
| Yes | 17( 54.8) | 19 (61.3) | 7 (38.9) | 49 (35.5)[&] | 92 (42.2) |
| No | 14(45.2) | 12 (38.7) | 11 (61.1) | 89 (64.5)[&] | 126 (57.8) |
| **Reported cough at admission**[&] | | | | | |
| Yes | 20 (64.5) | 9 (29.0) | 2 (11.1) | 28 (20.3)[&] | 59 (27.1) |
| No | 11 (35.5) | 22 (71.0) | 16 (88.9) | 110 (79.7)[&] | 159 (72.9) |

* Information was not available in three cases. # HIV status could not be ascertained in two cases (one in the group who died of TB, and the other among those with *M. tuberculosis* detection. [&]Fever or cough was not recorded in 5 cases. *M. tuberculosis* detection included patients in whom *M. tuberculosis* DNA was detected without histological evidence of TB

**Table 4.** Diagnostic performance of clinical diagnosis* to determine: a) cause of death b) concomitant TB c) TB disease at death (TB as cause of death + concomitant TB).

| | TB as cause of death* | | Concomitant TB# | | TB disease at death | |
|---|---|---|---|---|---|---|
| | % | 95%CI | % | ( 95% CI ) | % | 95%CI |
| Sensitivity | 19.4 | 7.5-37.5 | 19.4 | (7.5-37.5) | 32.26 | 18.0 - 49.8 |
| Specificity | 97.4 | 94.0-99.1 | 90.1 | (85.0 - 93.9) | 90.06 | 87.0 - 95.4 |
| PPV | 54.5 | 23.4-83.3 | 24 | (9.4 - 45.1) | 55.56 | 25.5 - 64.7 |
| NPV | 88.2 | 83.1-92.2 | 87.4 | (81.9 - 91.7) | 77.54 | 81.7 - 91.6 |

**PPV: positive predictive value; NPV: negative predictive value**

**Figure 1**

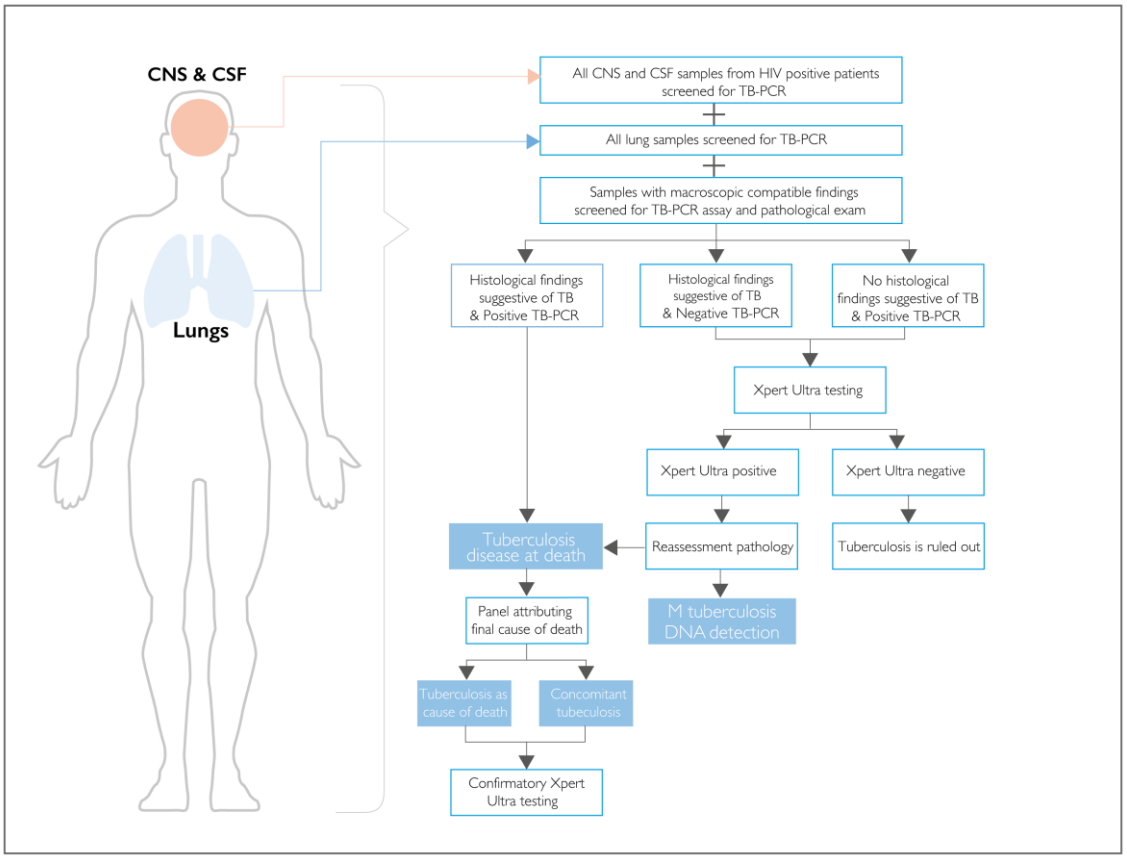

CNS & CSF
Lungs
All CNS and CSF samples from HIV positive patients screened for TB-PCR
All lung samples screened for TB-PCR
Samples with macroscopic compatible findings screened for TB-PCR assay and pathological exam
Histological findings suggestive of TB & Positive TB-PCR
Histological findings suggestive of TB & Negative TB-PCR
No histological findings suggestive of TB & Positive TB-PCR
Xpert Ultra testing
Xpert Ultra positive
Xpert Ultra negative
Tuberculosis disease at death
Reassessment pathology
Tuberculosis is ruled out
Panel attributing final cause of death
M tuberculosis DNA detection
Tuberculosis as cause of death
Concomitant tubeculosis
Confirmatory Xpert Ultra testing

**Figure 2**

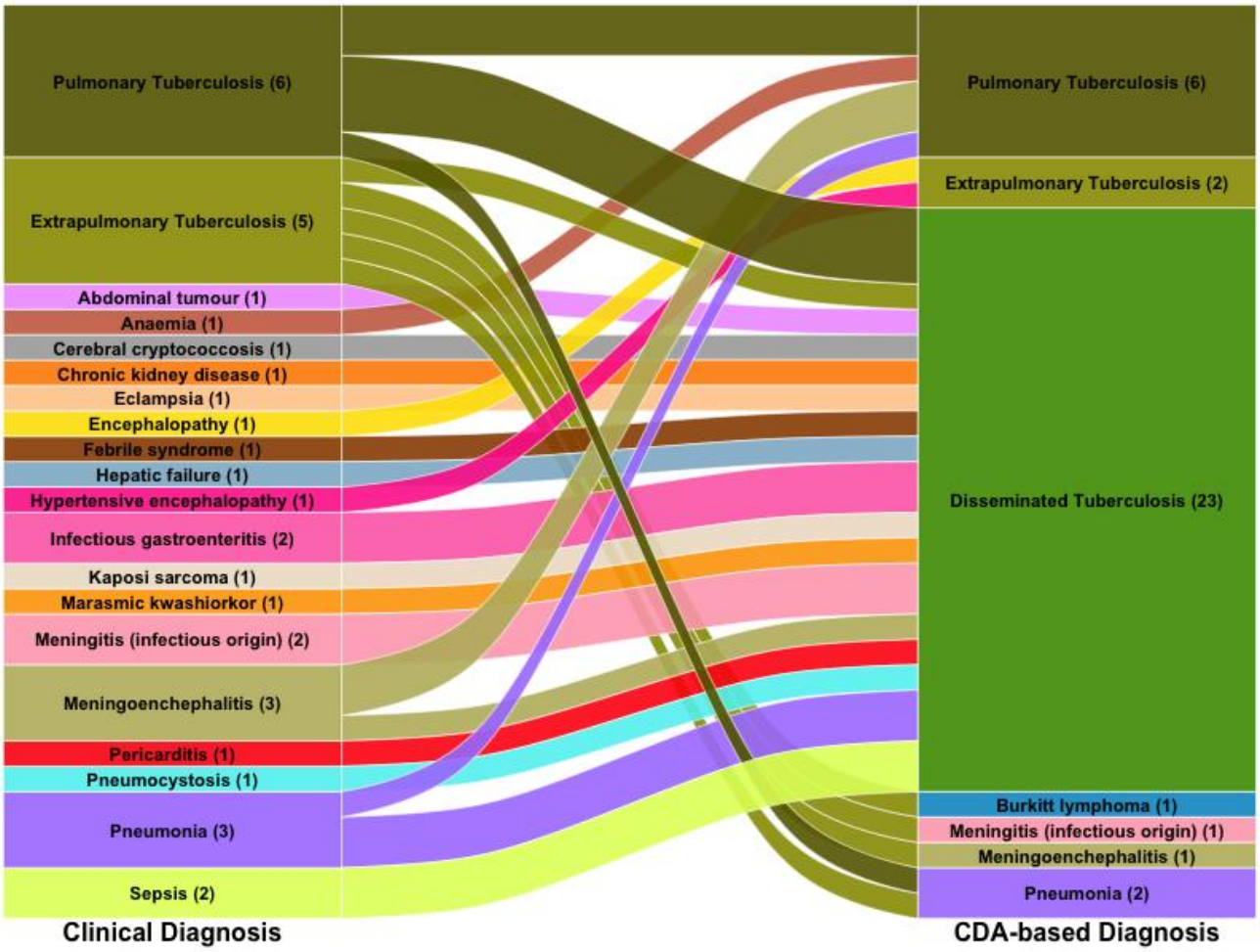
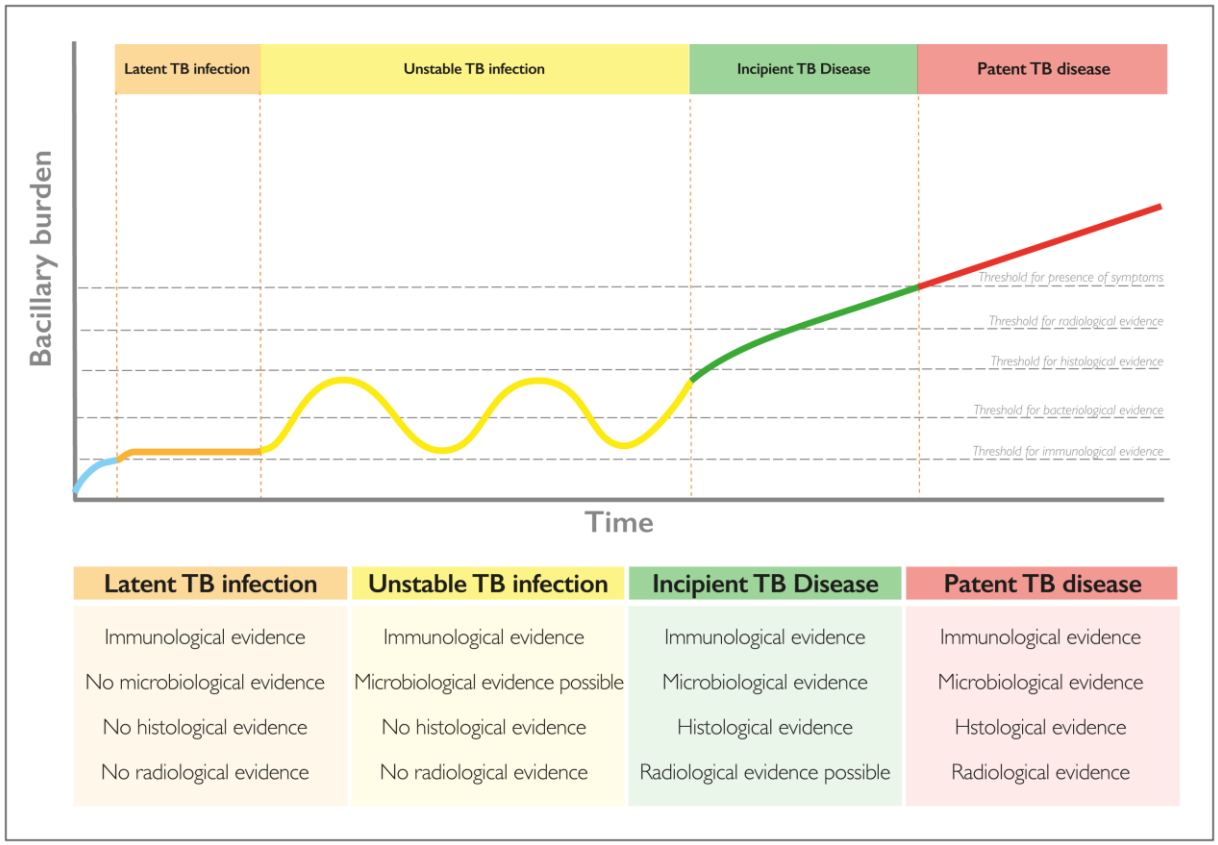

Pulmonary Tuberculosis (6)
Extrapulmonary Tuberculosis (5)
Abdominal tumour (1)
Anaemia (1)
Cerebral cryptococcosis (1)
Chronic kidney disease (1)
Eclampsia (1)
Encephalopathy (1)
Febrile syndrome (1)
Hepatic failure (1)
Hypertensive encephalopathy (1)
Infectious gastroenteritis (2)
Kaposi sarcoma (1)
Marasmic kwashiorkor (1)
Meningitis (infectious origin) (2)
Meningoenchephalitis (3)
Pericarditis (1)
Pneumocystosis (1)
Pneumonia (3)
Sepsis (2)
Clinical Diagnosis
Pulmonary Tuberculosis (6)
Extrapulmonary Tuberculosis (2)
Disseminated Tuberculosis (23)
Burkitt lymphoma (1)
Meningitis (infectious origin) (1)
Meningoenchephalitis (1)
Pneumonia (2)
CDA-based Diagnosis

**Figure 3**




| Latent TB infection | Unstable TB infection | Incipient TB Disease | Patent TB disease |
|---|---|---|---|
| Immunological evidence | Immunological evidence | Immunological evidence | Immunological evidence |
| No microbiological evidence | Microbiological evidence possible | Microbiological evidence | Microbiological evidence |
| No histological evidence | No histological evidence | Histological evidence | Hstological evidence |
| No radiological evidence | No radiological evidence | Radiological evidence possible | Radiological evidence |